cAMP ACTIVATED Cl^- AND K^+ CURRENTS IN SHARK RECTAL GLAND CELLS

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Forskolin and cpt-cAMP increase Cl^- currents in cells of the rectal gland of the spiny dogfish, Squalus acanthias, (Greger et al., Pflügers Arch. 402: 376-384, 1984; Devor et al., Am. J. Physiol., 268:C70-C79, 1995). These Cl^- currents pass through apical Cl^- channels corresponding to a dogfish homologue of the human cystic fibrosis transmembrane conductance regulator (CFTR). DFTR, the dogfish homologue, is 72 % homologous with CFTR at the protein level (Marshall et al., J. Biol. Chem. 266:22749-22754, 1991). Cl^- flowing out of the cell establishes a lumen-negative transepithelial potential difference with Na^+ then passively moving through the paracellular spaces to produce NaCl secretion (Greger et al., Pflügers Arch. 402: 376-384,1984). If the stimulation by secretory agonists resulted only in the activation of apical Cl^- channels, then the membrane potential would shift to the Cl^- equilibrium potential (E_{Cl}), and Cl^- efflux would not occur. Rather, an increase in K^+ channel activity hyperpolarizes the membrane potential to permit Cl^- efflux. Activation of both Cl^- and K^+ conductances by cAMP-dependent secretagogues is observed in airway, intestinal and other secretory cells (Devor and Frizzell, Am. J. Physiol. 265: C1271-C1280, 1993). In the present study, we used the perforated patch-clamp technique and primary cultures of spiny dogfish, Squalus acanthias, rectal gland (SRG) (Valentich and Forrest, Am. J. Physiol. 260: C813-823, 1991) to examine activation of Cl^- and K^+ conductances by cAMP secretagogues and a pharmacological activating agent (see below).

Figure 1A shows the response of one cell to stimulation by forskolin (10 μM) and cpt-cAMP (400 μM) during perforated whole-cell patch-clamp recording. cAMP stimulated an inward current (downward deflection) when the cell was voltage-clamped to E_K (-93 mV) and an outward current (upward deflection) when the cell was voltage-clamped to E_{Cl} (-29 mV); this reflects a stimulation of Cl^- and K^+ currents, respectively. Addition of charybdotoxin (CTX 30 nM) did not affect either the Cl^- or K^+ currents, whereas glibenclamide (300 μM) which inhibits the CFTR and K^+ channels in airway cells (Sheppard and Welsh, J. Gen. Physiol. 100: 573-591, 1992) inhibited both currents. Previously, we found no activation of K^+ conductance by cAMP in the conventional whole-cell patch-clamp configuration (Devor et al., Am. J. Physiol., 268:C70-C79, 1995). The use of nystatin permits whole-cell recordings under conditions where cell wash-out is prevented (Horn & Marty, J. Gen. Physiol 92: 145-159, 1988). This suggests that cAMP activation of the K^+ conductance pathway involves a diffusible second messenger, whose identify is currently unknown. The K^+ conductance activated by cAMP is not CTX sensitive, and therefore is presumably not similar to the Ca^{2+}-activated K^+ conductance of T84 cells (Devor and Frizzell, Am. J. Physiol. 265: C1271-C1280, 1993).